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Histone Deacetylase Inhibition Rescues Maternal Deprivation-Induced GABAergic Metaplasticity through Restoration of AKAP Signaling

Highlights

- Maternal deprivation (MD) did not affect AMPAR-mediated synaptic transmission in VTA DA neurons
- MD induced both pre- and postsynaptic GABAergic LTD
- MD induced GABAergic metaplasticity through disruption of AKAP150 signaling
- GABAergic synaptic and AKAP signaling deficits were rescued by HDAC inhibition

Authors

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In Brief

Childhood adversities increase the risk of later psychopathology via dopamine dysregulation. Authement et al. demonstrate that early maternal deprivation induced GABAergic metaplasticity in ventral tegmental area dopamine neurons through reversible epigenetic mechanisms that involve the scaffolding A-kinase anchoring protein (AKAP79/150).

Authement et al., 2015, Neuron *86*, 1240–1252
June 3, 2015
http://dx.doi.org/10.1016/j.neuron.2015.05.024

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Histone Deacetylase Inhibition Rescues Maternal Deprivation-Induced GABAergic Metaplasticity through Restoration of AKAP Signaling

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http://dx.doi.org/10.1016/j.neuron.2015.05.024

SUMMARY

Adverse early-life experiences such as child neglect and abuse increase the risk of developing addiction and stress-related disorders through alterations in motivational systems including the mesolimbic dopamine (DA) pathway. Here we investigated whether a severe early-life stress (i.e., maternal deprivation, MD) promotes DA dysregulation through an epigenetic impairment of synaptic plasticity within ventral tegmental area (VTA) DA neurons. Using a single 24-hr episode of MD and whole-cell patch clamp recording in rat midbrain slices, we show that MD selectively induces long-term depression (LTD) and shifts spike timing-dependent plasticity (STDP) toward LTD at GABAergic synapses onto VTA DA neurons through epigenetic modifications of postsynaptic scaffolding A-kinase anchoring protein 79/150 (AKAP79/150) signaling. Histone deacetylase (HDAC) inhibition rescues GABAergic metaplasticity and normalizes AKAP signaling in MD animals. MD-induced reversible HDAC-mediated GABAergic dysfunction within the VTA may be a mechanistic link for increased propensity to mental health disorders following MD.

INTRODUCTION

Child neglect and abuse are pervasive and costly public health concerns with serious negative long-term effects on child health and development, including an increased risk of mental health disorders and substance abuse in later life (Rentesi et al., 2013). Understanding the consequences of child abuse and neglect on neural processes that shape memories and guide behaviors is critical for developing psychological and pharmacological interventions to improve outcomes of these children. Synaptic plasticity (changes in synaptic strength) is an experience-dependent learning mechanism of the brain used to process and store information vital for survival. However, prolonged exposure to stress and addictive drugs dysregulates synaptic plasticity within mesocorticolimbic brain systems (i.e., the brain reward pathway), thereby shaping pathological learning of anxiety-, depressive-, and addictive-like behaviors (Sinha, 2008). A single 24-hr episode of early maternal deprivation (MD) in rodents has been widely used to model severe early-life stress such as child abuse and neglect. This model was shown to promote behavioral impairments with disturbances in stress responsiveness that resemble anxiety-, depressive-, psychotic-, and addictive-like symptoms (Faturi et al., 2010; Gruss et al., 2008; Kember et al., 2012; Marco et al., 2009; Nishi et al., 2014; Roceri et al., 2002). During MD, the hypothalamic-pituitary-adrenal stress response is persistently upregulated and the ability of MD animals to cope with stressful situations is significantly diminished, with symptoms evident from adolescence and into adulthood (Marco et al., 2009). Moreover, MD rodents and adolescent human subjects reporting low parental care show heightened impulsivity and exhibit anxiety-like behaviors associated with increased levels of monoamine neurotransmitters including DA in the mesocorticolimbic dopaminergic pathway (Ellenbroek et al., 2005; Hall et al., 1999; Llorente et al., 2010; Pruessner et al., 2004; Rentesi et al., 2013).

The mesocorticolimbic dopaminergic pathway originating from the ventral tegmental area (VTA) controls mood and motivation. DA release from the VTA is a key detector of reward response and is involved in reward-related learning. Dysregulation of DA signaling from the VTA has been linked to neuropsychiatric disorders. However, whether early MD affects the reward learning processes and synaptic function in the VTA is unknown. Strengthening (long-term potentiation, LTP) and weakening (long-term depression, LTD) of excitatory and inhibitory synapses onto VTA DA neurons critically influences DA cell firing and release (Dacher and Nugent, 2011b). Investigations are currently aiming to identify patterns of learning-related DA neuronal activity and signaling mechanisms that drive activity-dependent plasticity of VTA DA neurons. Such knowledge will lead to a better understanding of the function DA neurons serve in reward-related behaviors and how the brain reward circuitry is remodeled by stress and addictive drugs. There is an increasing interest in the functional roles of synaptic plasticity at inhibitory GABAergic synapses in brain circuits, particularly the brain reward pathway, where GABAergic dysfunction significantly contributes to the pathophysiology of depression, addiction, anxiety, and schizophrenia (Dacher and Nugent, 2011b; Gao and Bao, 2011).

Epigenetic mechanisms regulate gene expression without altering the DNA sequence. Many of these are activated by early environmental experiences and shape neuronal plasticity, and hence behavior. Epigenetic modifications in the VTA such as DNA methylation are critical for the formation of reward learning (Day et al., 2013). Chromatin modifications by enzymes such as histone deacetylases (HDACs) also alter gene expression in neurons and neuroplasticity underlying memory formation (Abel and Zukin, 2008; Haggarty and Tsai, 2011). $GABA_A$ receptors ($GABA_AR$) are epigenetically regulated in response to early-life stress and addictive drugs (Arora et al., 2013; Kennedy et al., 2013). $GABA_AR$ signaling in DA neurons plays a key role in reward-motivated learning (Laviolette and van der Kooy, 2001; Parker et al., 2011). However, it is unknown which epigenetic mechanisms influence GABAergic plasticity and mediate maladaptive forms of such plasticity in the VTA. Because of the importance of $GABA_AR$ signaling in reward learning and the critical implications of epigenetic regulation of memory, stress-related disorders, schizophrenia, and addiction (Abel and Zukin, 2008; Costa et al., 2003; Rudenko and Tsai, 2014), we hypothesized that MD would trigger HDAC-mediated epigenetic changes in the gene encoding the scaffold protein AKAP150 (human 79/rodent 150, also referred to as AKAP5) in the VTA. AKAP150 is an important integrator of signaling molecules to glutamatergic and GABAergic synapses that control the synaptic trafficking underlying synaptic plasticity (Dacher et al., 2013; Jurado et al., 2010; Lu et al., 2007, 2008; Sanderson et al., 2012). Epigenetic changes leading to altered AKAP signaling would in turn selectively reduce GABAergic inhibition in the VTA and thus contribute to the development of later psychopathology following MD. Indeed, AKAP proteins are epigenetically regulated (Choi et al., 2004; Reissner et al., 2011), and global knockout of AKAP150 is associated with synaptic and behavioral abnormalities (Tunquist et al., 2008). Therefore, we tested whether a single 24-hr episode of MD, on postnatal day 9 (P9), would affect synaptic function and plasticity of VTA DA neurons through reversible epigenetic modifications of genes involved in AKAP signaling and learning mechanisms in the VTA. Our results suggest that MD induces an aberrant GABAergic metaplasticity through reversible epigenetic mechanisms that alter the AKAP signaling associated with memory formation in the VTA.

RESULTS

Repeated or prolonged exposure to stress increases DA levels in projection areas of the VTA, leading to heightened impulsivity and anxiety-like behaviors from adolescence to adulthood (Meaney et al., 2002). MD-induced changes in synaptic transmission onto VTA DA neurons are likely to occur but have yet to be described. Stress effectively modulates synaptic plasticity at both glutamatergic and GABAergic synapses onto DA neurons (Niehaus et al., 2010; Saal et al., 2003); thus, we first tested whether MD induces an excitatory LTP in VTA DA neurons.

MD Did Not Alter Glutamatergic Synaptic Transmission and Plasticity of VTA DA Neurons

Although acute stress potentiates glutamatergic synapses onto VTA DA neurons (Saal et al., 2003), we found that α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor (AMPAR)/N-methyl-D-aspartate receptor(NMDAR) ratios (AMPAR/NMDAR ratios) (commonly used as a reliable measurement for glutamatergic plasticity) measured in VTA DA neurons from 14- to 21-day-old non-MD and MD rats showed no significant difference (Figures 1A–1C, non-MD, n = 8: 0.86 ± 0.23; MD, n = 9: 0.81 ± 0.20; unpaired Student's t test: p = 0.87501). To further test whether MD induced changes in the function and/or surface expression of AMPARs (postsynaptic plasticity) or glutamate release from glutamatergic terminals (presynaptic plasticity) onto VTA DA neurons, we recorded AMPAR-mediated miniature EPSCs (mEPSCs) in the presence of the sodium channel blocker tetrodotoxin (TTX, 1 μM) in slices prepared from non-MD and MD rat pups. We found that the average and cumulative probability of AMPAR-mediated mEPSC amplitudes and frequencies did not differ between MD and non-MD rats, suggesting that MD did not change AMPAR function in VTA DA neurons (Figures 1F–1K, non-MD, n = 8: 13.47 ± 0.7 pA, 3.848 ± 0.36 Hz; MD, n = 9: 12.75 ± 0.59 pA, 3.249 ± 0.36 Hz; unpaired Student's t tests: p = 0.19573 for amplitude, p = 0.12262 for frequency; Kolmogorov-Smirnov [KS] tests for cumulative distribution curves: p = 0.927 for amplitude, p = 0.989 for frequency). MD alters AMPAR and NMDAR subunit composition in the hippocampus (Pickering et al., 2006; Roceri et al., 2002; Rodenas-Ruano et al., 2012), and given that such changes in AMPAR subunit composition could occur without alteration in overall synaptic strength measured by mEPSC amplitude or AMPAR/NMDAR ratios, we recorded evoked AMPAR-mediated EPSCs at different holding potentials while intracellularly applying spermine. This allowed us to detect the relative contribution of inwardly rectifying Ca^{2+}-permeable/GluA2-lacking AMPARs to synaptic transmission. AMPAR rectification was unaltered after MD, and EPSC IV plots were linear in both groups. This is consistent with the presence of GluA2 in most synaptic AMPARs in MD rats, similar to non-MD rats (Figures 1D and 1E, AMPAR rectification indices: non-MD, n = 5: 1.444 ± 0.23; MD, n = 5: 1.4677 ± 0.33; unpaired Student's t test: p = 0.9548; two-way ANOVA for IV curves, $F_{(1,5)} = 0.01102$, p = 0.92047).

MD Induced Pre- and Postsynaptic LTD at GABAergic Synapses onto VTA DA Neurons

Acute stress was previously shown to block a presynaptic form of LTP at GABAergic synapses onto VTA DA neurons (Niehaus et al., 2010; Nugent et al., 2007). We tested the effects of MD on $GABA_AR$-mediated miniature IPSCs (mIPSCs) to identify whether MD per se induced any form of GABAergic plasticity. We found that the frequency and amplitude of mIPSCs were significantly reduced in MD compared to non-MD rats. (Figures 2A and 2B, non-MD, n = 6: 30.25 ± 0.2.44 pA, 4.92 ± 0.38 Hz; MD, n = 8: 22.33 ± 1.06 pA, 3.42 ± 0.45 Hz; unpaired Student's t tests: p = 0.0067 for amplitude, p = 0.0316 for frequency; KS tests for cumulative distribution curves: p < 0.0001 for amplitude, p = 0.036 for frequency). These findings suggest that MD suppressed both pre- and postsynaptic function of GABAergic synapses onto VTA DA neurons.

MD Shifted Spike Timing-Dependent Plasticity at GABAergic Synapses onto DA Neurons toward LTD

Previous experiences such as in vivo exposure to addictive drugs, stress, and sensory or visual deprivation can change the ability of

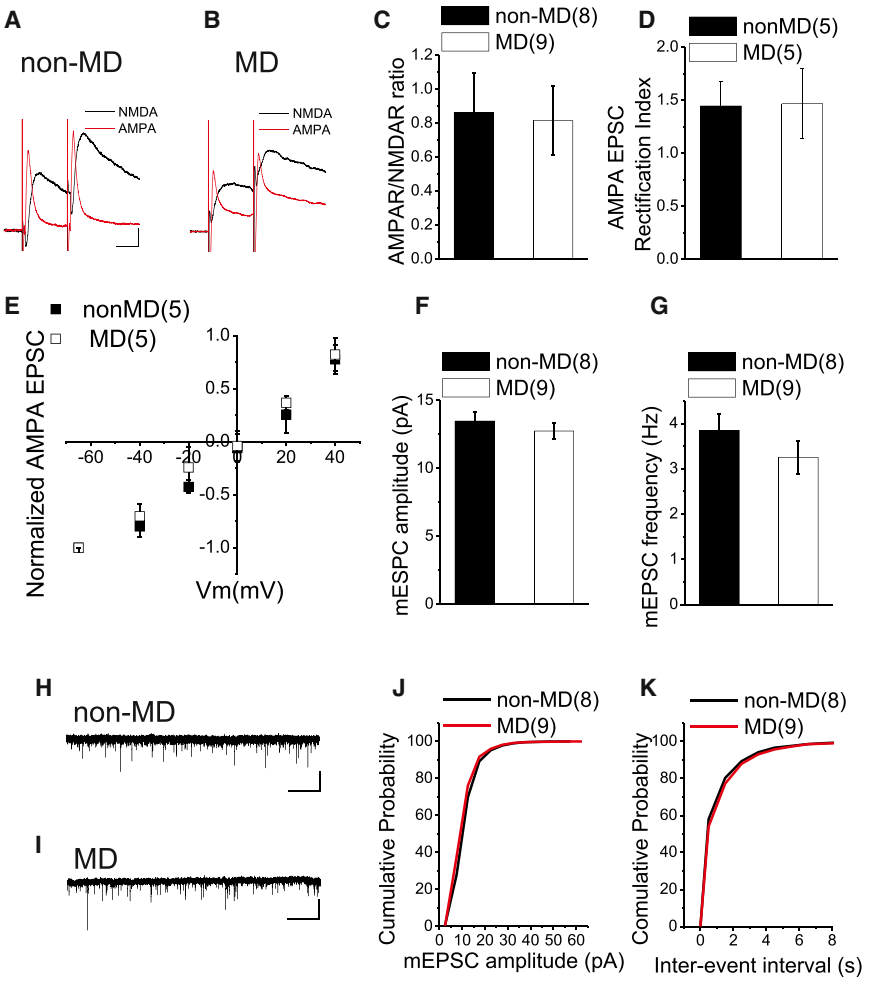

Figure 1. MD Did Not Alter Basal Excitatory Synaptic Function or AMPAR Subunit Composition in VTA DA Neurons

(A and B) Sample traces of AMPA (red) and NMDA (black) EPSCs recorded at +40 mV from DA neurons of non-MD and MD rat pups (calibration bars: 50 pA/25 ms).

(C) Summary of AMPAR/NMDAR ratios obtained from non-MD and MD animals.

(D) MD did not change AMPAR EPSC rectification.

(E) Normalized I-V plots of AMPAR EPSCs in VTA DA neurons of non-MD and MD rat pups. In both (D) and (E), AMPAR EPSC recordings were performed with intra-pipette inclusion of spermine.

(F and G) Average bar graphs of mEPSC amplitude and frequency from non-MD and MD rats.

(H and I) Sample AMPAR-mediated mEPSC traces from non-MD and MD rats (calibration bars: 20 pA/5 s).

(J and K) Cumulative probability plots of amplitude and frequency (inter-event interval) for all mEPSCs in non-MD and MD rats.

In this and all subsequent figures, numbers within the figure legends indicate the number of animals examined. Error bars represent SEM.

synapses to undergo subsequent plasticity in response to LTP and LTD induction protocols. This phenomenon is referred as metaplasticity (Abraham and Bear, 1996). Although traditional induction protocols of LTP and LTD have been successfully used to induce synaptic plasticity, near-coincidental pre- and postsynaptic firing can also trigger a form of plasticity where the precise timing and patterns of spikes are critical (i.e., spike timing-dependent plasticity, STDP). This is an attractive induction protocol since it mimics naturally occurring neuronal activity required for plasticity (Caporale and Dan, 2008). Recently, we showed that GABAergic synapses onto VTA DA neurons were capable of exhibiting a bidirectional postsynaptic Hebbian STDP (i.e., STD-LTP_{GABA} and STD-LTD_{GABA} in response to repeated pre-post spike pairings at positive interval of +15 ms and post-pre spike pairings at negative interval of −5 ms, respectively). Interestingly, this STDP was triggered by correlated activities of the presynaptic glutamatergic input and postsynaptic DA cells where NMDARs served as coincident detectors for this heterosynaptic plasticity (Kodangattil et al., 2013). Given that early MD is a potent stressor, we hypothesized that MD induces metaplastic changes in GABAergic synapses that subsequently alter the response of DA neurons to STDP protocols. Therefore, we used STDP protocols to induce GABAergic STDP in midbrain slices prepared from non-MD and MD rats. To define the temporal window for STDP induction, we tested three additional STDP protocols with different time intervals (−15 ms, +5 ms, and +25 ms). Data for time courses of STDP at these three additional intervals are shown in Figure S1, and the averaged magnitudes of synaptic change for all STDP experiments with a given Δt are depicted in Figure 3E. Pairings at a negative interval of −15 ms and a positive interval of +25 ms did not induce any form of plasticity in slices from non-MD or MD rats, demonstrating a short temporal window for GABAergic STDP in the VTA. Interestingly, a robust STD-LTD_{GABA} was induced in response to pre-post positive pairing at +5 ms intervals in slices from MD rats while non-MD rats did not exhibit STDP at this time interval. Moreover, slices prepared from MD animals did not exhibit STD-LTP_{GABA} at +15 ms (in fact, the polarity of STDP in response to pre-post pairings at this interval shifted toward LTD), whereas non-MD rats showed robust STD-LTP_{GABA}. STD-LTD_{GABA} (induced in response to negative pairings at −5 ms) was also absent in slices from MD rats, whereas plasticity was intact in slices from non-MD rats. Altogether these data suggest that MD-induced metaplastic changes impaired the ability of GABAergic synapses to exhibit normal bidirectional STDP, narrowed the STDP window, and shifted STDP toward LTD. In Figure 3, the time courses of STDP are only shown at the negative timing of −5 ms and positive timing of +15 ms. In the remainder of the paper, only these two intervals are used for STDP induction and are designated as "pre-post" for positive timing of +15 ms and "post-pre" for negative timing of −5 ms (Figures 3A and 3D, for STD-LTD_{GABA}, non-MD, n = 6: 64% ± 2.4% of pre-STDP values, $F_{11.53,57.67}$ = 11.09, p < 0.0001, MD, n = 7: 96% ± 2.7%

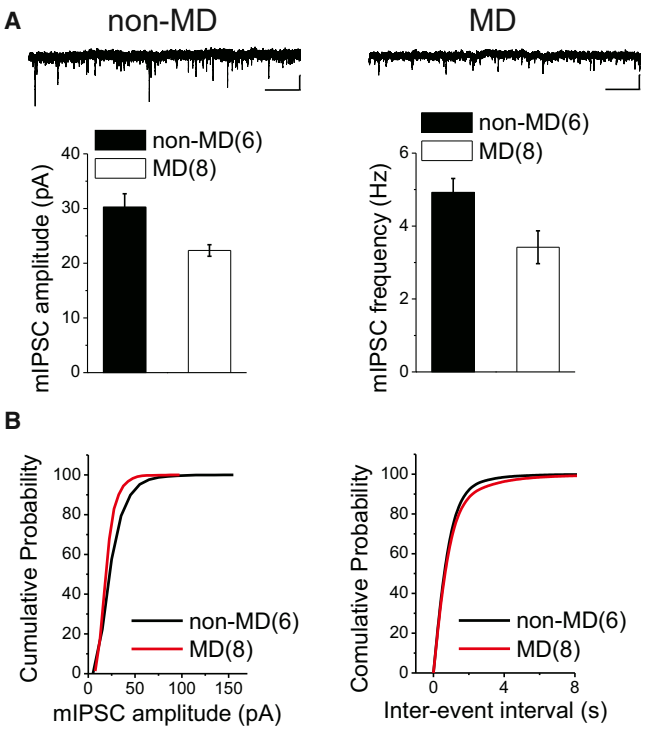

Figure 2. MD Induced Pre- and Postsynaptic LTD at GABAergic Synapses onto VTA DA Neurons

(A) Sample $GABA_AR$-mediated mIPSC traces from non-MD and MD rats (top) and average bar graphs of mIPSC amplitude and frequency from non-MD and MD rats (bottom) (calibration bars: 20 pA/5 s).

(B) Cumulative probability plots of amplitude and frequency (inter-event interval) for all mIPSCs in non-MD and MD rats.

Error bars represent SEM.

of pre-STDP values, $F_{9.43,\ 37.73} = 0.478$, $p = 0.886$, unpaired Student's t test for STD-LTD_{GABA} between MD and non-MD rats, $p < 0.001$; Figures 3B and 3C for STD-LTP_{GABA}: non-MD, n = 8: 151% ± 8.4% of pre-STDP values, $F_{5.26,36.87} = 3.369$, $p = 0.012$, MD, n = 8: 85% ± 21% of pre-STDP values, $F_{3.32,23.26} = 3.368$, $p = 0.032$, unpaired Student's t test for STD-LTP_{GABA} between MD and non-MD rats, $p < 0.001$; Figure 3E shows the temporal windows of STDP in non-MD and MD rats).

GABAergic STDP in the VTA Required the PKA-AKAP-Calcineurin Signaling Complex

Although we identified a shift in GABAergic STDP toward LTD following MD, the cellular and molecular mechanisms underlying this metaplasticity remained unknown. To identify potential mechanisms of metaplasticity, we first characterized STDP. Previously we discovered that the AKAP150 complex selectively controlled $GABA_AR$ trafficking-mediated GABAergic plasticity in the VTA. Postsynaptic disruption of Protein Kinase A (PKA)-AKAP association or PKA inhibition induced a calcineurin (CaN)-dependent, long-lasting reduction in synaptic $GABA_A$ responses that mimicked LTD_{GABA} (induced in response to a traditional LTD pairing protocol) without affecting AMPAR-mediated glutamatergic transmission in VTA DA neurons (Dacher et al., 2013; Dacher and Nugent, 2011a). Here, we determined whether the PKA-AKAP-CaN complex also underlies GABAergic STDP. Consistent with our previous results, intra-pipette AKAP inhibitor Ht31 (1 μM) resulted in a remarkably rapid rundown of synaptic transmission, whereas control peptide Ht31p (1 μM) did not (Figure 4A, Ht31p cells, n = 19: 93% ± 4% of the first 5-min baseline values, $F_{10.387,\ 46.161} = 1.035$, $p = 0.44$; Ht31 cells, n = 14: 58% ± 1% of the first 5-min baseline values, $F_{15.902,\ 47.707} = 6.614$, $p < 0.00001$). Once Ht31-induced depression plateaued, STDP protocols were used to trigger STD-LTP_{GABA} or STD-LTD_{GABA}. Intra-pipette Ht31 prevented the induction of STD-LTP_{GABA}, while Ht31p-filled cells expressed STD-LTP_{GABA} (Figures 4B and 4C, Ht31p cells, n = 7: 140% ± 2% of pre-STDP values, $F_{10.127,9.922} = 6.124$, $p < 0.0001$; Ht31 cells, n = 7: 99.3% ± 2.8% of pre-STDP values, $F_{11.78,47.151} = 0.93$, $p = 0.453$). Moreover, intra-pipette Ht31-induced rundown occluded STD-LTD_{GABA}, whereas Ht31p did not affect the plasticity (Figure 5A, Ht31p cells, n = 12: 71% ± 2% of pre-STDP values, $F_{9.192,\ 45.962} = 6.316$, $p < 0.0001$; Ht31 cells, n = 7: 105% ± 3% of pre-STDP values, $F_{14.945,\ 59.779} = 0.878$, $p = 0.591$), suggesting that the disruption of association between PKA and AKAP is sufficient to induce STD-LTD_{GABA}. Consistent with our previous results, postsynaptic inhibition of PKA by intracellular inclusion of $PKI_{(6-22)}$ (10 μM), a membrane-impermeant PKA inhibitor, significantly reduced IPSC amplitude (Figures 4D and 4E; PKI cells, n = 6: 63% ± 2% of the first 5-min baseline values, $F_{11.37,45.48} = 21.145$, $p < 0.0001$). $PKI_{(6-22)}$-induced depression of IPSCs not only inhibited STD-LTP_{GABA} but also shifted the polarity of plasticity toward LTD (Figures 4E and 4F; control STD-LTP for $PKI_{(6-22)}$, n = 7: 121% ± 1.4% of pre-STDP values, $F_{8.58,34.34} = 3.356$, $p = 0.005$; $PKI_{(6-22)}$ cells, n = 6: 76.6% ± 1.1% of pre-STDP values, $F_{16.95,50.86} = 3.147$, $p = 0.01$). This suggests that PKA association with AKAP150 promotes LTP and may control the polarity of STDP. We also confirmed that, similar to LTD_{GABA} induced by our LTD pairing protocol (Dacher et al., 2013), STD-LTD_{GABA} was postsynaptic, was AKAP- and CaN-dependent, and involved clathrin-mediated endocytosis. We attempted to induce STD-LTD_{GABA} induction in the presence of intra-pipette CaN inhibitors (1 μM cyclosporin A or FK506). Both CaN inhibitors blocked STD-LTD_{GABA} (Figure 5C, control STD-LTD_{GABA} for cyclosporin A, n = 5: 60% ± 0.5% of pre-STDP values, $F_{8.94,26.84} = 7.026$, $p < 0.0001$; cyclosporin A cells, n = 6: 98% ± 1.3% of pre-STDP values, $F_{7.5,15} = 0.870$, $p = 0.557$; Figure 5D, control STD-LTD_{GABA} for FK506, n = 6: 61 ± 2.8% of pre-STDP values, $F_{7.92,31.69} = 6.277$, $p < 0.0001$; FK506 cells, n = 4: 92% ± 2.5% of pre-STDP values, $F_{14.84,44.54} = 0.663$, $p = 0.830$). Postsynaptic inhibition of clathrin was achieved by loading cells with 50 μM Pitstop2 and prevented STD-LTD_{GABA}, whereas cells loaded with 50 μM Pitstop2 control peptide still exhibited STD-LTD_{GABA} (Figure 5B, control peptide cells, n = 6: 68% ± 1% of pre-STDP values, $F_{7.38,29.15} = 6.592$, $p < 0.0001$; Pitstop2 cells, n = 6: 109.5% ± 1.1% of pre-STDP values, $F_{7.98,23.95} = 1.210$, $p = 0.352$).

MD Disrupted the AKAP150 Signaling Complex at GABAergic Synapses in the VTA

Since AKAP150 signaling was implicated in $GABA_AR$-mediated plasticity in the VTA (including STDP here, Figures 4 and 5) and MD affected GABAergic transmission and plasticity without affecting AMPAR-mediated glutamatergic transmission (Figures

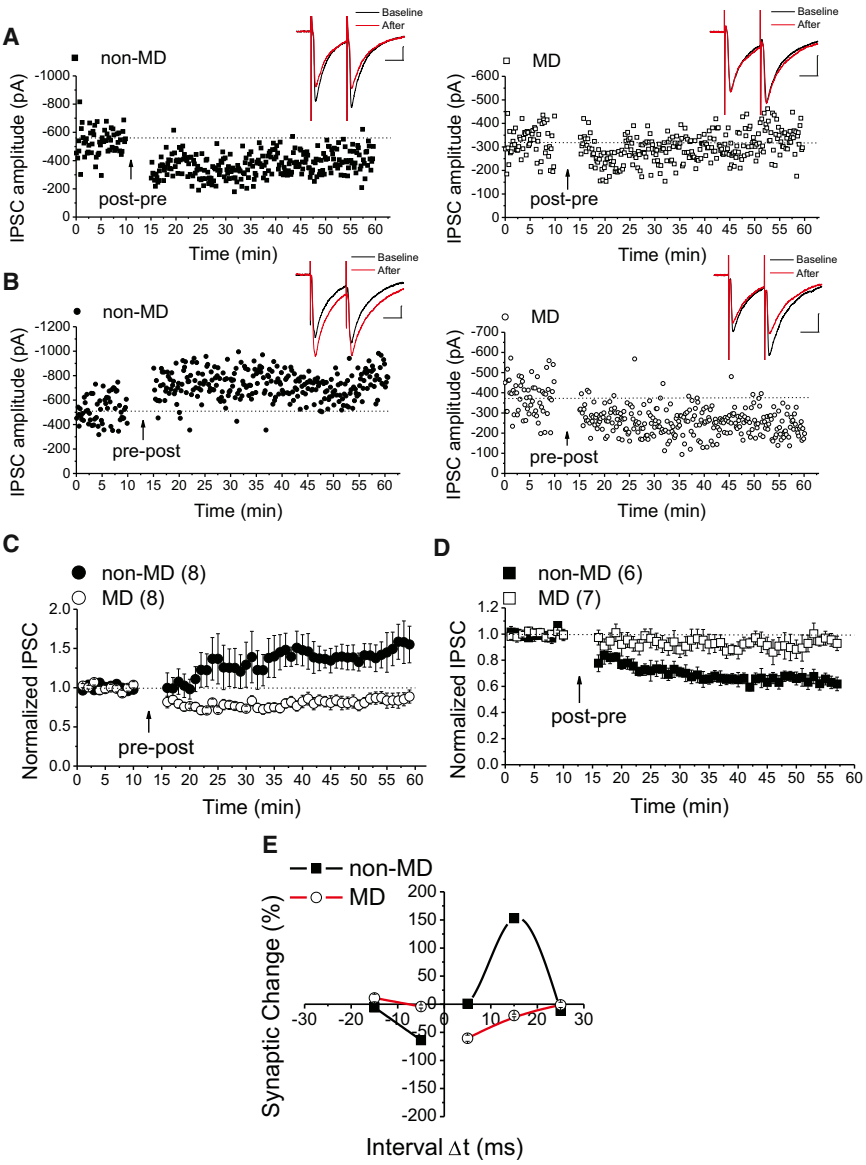

Figure 3. MD Triggered Metaplasticity of GABAergic STDP in VTA DA Neurons

(A and B) Single experiments showing induction of STDP in response to pre-post (+15 ms) and post-pre (−5 ms) STDP protocols in slices from non-MD and MD rats. At the arrow, STDP was induced using STDP protocols. Insets: averaged IPSCs before (black) and 25 min after STDP protocol (red). In this and all figures, ten consecutive traces from each condition were averaged for illustration as inset. Calibration: 100 pA, 25 ms.

(C and D) Average experiments from DA neurons of non-MD (filled symbols) and MD (open symbols) rats in response to STDP protocols.

(E) Summary of STDP induction with various time interval (Δt) STDP protocols in non-MD and MD rats. Each data point represents the averaged STDP levels for all experiments with a given Δt. Error bars represent SEM.

1, 2, and 3), we assumed that MD-induced metaplasticity (Figure 3) involved AKAP signaling. Similarly to the experiments shown in Figure 4A, we attempted to induce chemical LTD_{GABA} using intra-pipette Ht31 in slices from MD and non-MD animals. Intra-pipette Ht31 did not induce a significant rundown of IPSCs in cells recorded from MD animals, whereas cells recorded from non-MD animals showed a robust and rapid rundown of IPSCs. Control Ht31p-filled cells recorded from MD or non-MD rats did not show any rundown of IPSCs (Figures 6A and 6B, non-MD: Ht31p cells, n = 7, 96% ± 3% of the first 5-min baseline values, $F_{18.37,36.75} = 1.474$, p = 0.155; Ht31 cells, n = 7, 67% ± 2.8% of the first 5-min baseline values, $F_{4.36,\ 13.079} = 13.938$, p < 0.0001; Figures 6A and 6C, MD rats: Ht31p cells, n = 7, 101% ± 4% of the first 5-min baseline values, $F_{3.67,14.678} = 0.377$, p = 0.807; Ht31 cells, n = 9, 91% ± 5.8% of the first 5-min baseline values, $F_{5.468,32.808} = 1.442$, p = 0.232, unpaired Student's t test for Ht31-induced LTD between MD and non-MD rats, p < 0.001). Furthermore, we confirmed that the LTD induced by pre-post protocol in MD animals was indeed STD-LTD_{GABA} and CaN dependent. Intra-pipette cyclosporin A (1 μM) prevented the induction of LTD in response to pre-post protocols (+15 ms) in MD animals but did not rescue STD-LTP_{GABA} (Figure S2), suggesting that MD-induced metaplastic disruption of AKAP150 signaling blocked the induction of STD-LTP_{GABA} and facilitated the induction of STD-LTD_{GABA}.

HDAC Inhibition Reversed MD-Induced GABAergic Metaplasticity through Restoration of Normal AKAP Signaling

HDACs contribute to the formation of condensed and transcriptionally silenced chromatin, which decreases gene transcription. Blocking histone deacetylation using HDAC inhibitors (HDACi) has been shown to improve learning, memory, and synaptic plasticity in rodents, indicating that site- and cell-specific HDAC inhibition may provide a selective and promising target for enhancing memory (Abel and Zukin, 2008; Haggarty and Tsai, 2011). Interestingly, adverse experiences in early life such as MD and low maternal care are also associated with long-lasting changes in the expression of critical synaptic plasticity- and GABAergic signaling-associated genes through chromatin remodeling (Bagot et al., 2012; Rodenas-Ruano et al., 2012; Zhang et al., 2010). Therefore, we sought to determine whether MD-induced GABAergic metaplasticity and impairment of AKAP signaling involve HDACs. We first tested whether local HDAC inhibition in the VTA (via in vitro incubation of midbrain slices in HDACi or vehicle) could rescue the GABAergic synaptic abnormalities that we detected in mIPSC recordings from slices of

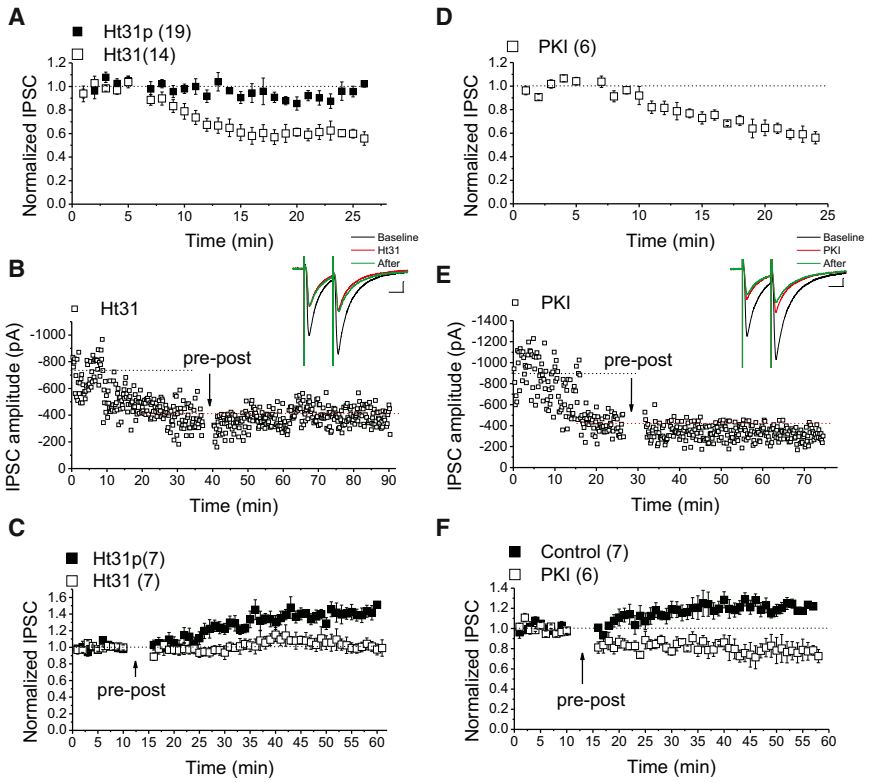

Figure 4. The Induction of STD-LTP$_{GABA}$ Required the Association of PKA with AKAP150

(A–F) Only non-MD rats were used for this set of experiments. (A) Averaged experiments with intra-pipette 1 μM Ht31 (open symbols) or intra-pipette of 1 μM Ht31p (filled symbols). (B) Single experiment illustrating the effect of intra-pipette Ht31 on basal synaptic transmission and the induction of STD-LTP$_{GABA}$ in the Ht31 loaded cell (only the last 5 min of the baseline responses before the emergence of the Ht31-induced depression is shown in this sample experiment). Inset: averaged IPSCs before (black) or the peak response of Ht31 (red) or 25 min after pre-post protocol (green). Calibration: 100 pA, 25 ms. (C) Averaged STD-LTP$_{GABA}$ experiments with intra-pipette Ht31 (open symbols) or Ht31p (filled symbols). (D) Averaged experiments with intra-pipette 10 μM PKI$_{(6-22)}$ (open symbols). (E) Single experiment illustrating the effect of intra-pipette PKI$_{(6-22)}$ on basal synaptic transmission and the induction of STD-LTP$_{GABA}$ in the PKI$_{(6-22)}$-loaded cell (only 5 min of the baseline before the emergence of the PKI$_{(6-22)}$-induced depression is shown in this sample experiment). Inset: averaged IPSCs before (black) or the peak response of PKI$_{(6-22)}$ (red) or 25 min after pre-post protocol (green). Calibration: 100 pA, 25 ms. (F) Averaged STD-LTP$_{GABA}$ experiments with (open symbols) or without (filled symbols) intra-pipette PKI$_{(6-22)}$. Error bars represent SEM.

MD animals (MD-induced GABAergic LTD). We used a selective class I HDACi (CI-994, 20 μM) that has previously been shown to attenuate remote fear memories (Gräff et al., 2014). Consistent with our previous findings (Figure 2), significant reductions in the average amplitude and frequency of mIPSCs were detected in DA cells from vehicle (DMSO)-incubated non-MD and MD slices (Figure 7, non-MD + DMSO, n = 6: 38.89 ± 5.58 pA, 5.39 ± 1.13 Hz; MD + DMSO, n = 6: 23.54 ± 2.55 pA, 3.55 ± 0.36 Hz; unpaired Student's t tests: p = 0.0023 for amplitude, p = 0.018 for frequency). A 2- to 4-hr in vitro incubation of midbrain slices prepared from MD animals in CI-994 reversed these GABAergic abnormalities (Figure 7B, MD + DMSO, n = 6: 23.54 ± 2.55 pA, 3.55 ± 0.36 Hz; MD + CI-994, n = 9: 31.52 ± 1.83 pA, 5.88 ± 0.93 Hz; unpaired Student's t tests: p = 0.021 for amplitude, p = 0.042 for frequency; KS tests for cumulative distribution curves: p < 0.0001 for amplitude, p < 0.0001 for frequency). CI-994 significantly reduced the amplitude and frequency of mIPSCs in HDACi-incubated midbrain slices from non-MD rats, although no significant difference between the average frequency of mIPSCs was detected after HDACi treatment (Figure 7A, non-MD + DMSO, n = 6: 38.89 ± 5.58 pA, 5.39 ± 1.13 Hz; non-MD + CI-994, n = 8: 28.63 ± 1.38 pA, 4.74 ± 0.47 Hz; unpaired Student's t tests: p = 0.045 for amplitude, p = 0.568 for frequency; KS tests for cumulative distribution curves: p < 0.0001 for amplitude, p = 0.043 for frequency).

Next we investigated the effects of in vitro HDAC inhibition on MD-induced metaplasticity of STDP using CI-994 and a widely used HDACi, sodium butyrate (NaB, 300 μM) (Levenson et al., 2004). We found that 2- to 4-hr in vitro incubation of midbrain slices prepared from MD animals in either NaB or CI-994 also recovered STDP to normal levels in these slices (Figure 8B, STD-LTD$_{GABA}$: MD, n = 7: 96% ± 2.7% of pre-STDP values, $F_{9.43,\ 37.73}$ = 0.478, p = 0.886, MD + NaB, n = 7: 73% ± 2% of pre-STDP values, $F_{9.705,48.523}$ = 8.523, p < 0.0001; Figure 8D, MD + DMSO, n = 4: 108% ± 2.7% of pre-STDP values, $F_{3.96,104}$ = 0.013, p = 0.765, MD + CI-994, n = 6: 63% ± 2.4% of pre-STDP values, $F_{3.86,19.32}$ = 7.255, p < 0.001; Figure 8F, STD-LTP$_{GABA}$: MD rats, n = 8: 85% ± 2.1% of pre-STDP values, $F_{3.32,23.26}$ = 3.368, p = 0.032, MD + NaB, n = 8: 150% ± 6.5% of pre-STDP values, $F_{12.19,24.39}$ = 4.01, p < 0.001; Figure 8H, MD + DMSO, n = 6: 65% ± 0.5% of pre-STDP values, $F_{29.8,119.25}$ = 7.044, p < 0.0001, MD + CI-994, n = 4: 142% ± 2% of pre-STDP values, $F_{21.72,43.45}$ = 3.144, p < 0.001). HDAC inhibitors had very different effects on VTA GABAergic synapses in slices from non-MD versus MD animals. Although these drugs inhibited GABAergic transmission and disrupted STDP in non-MD animals, synaptic transmission and plasticity in MD animals were normalized (Figure 8A, STD-LTD$_{GABA}$: non-MD, n = 6: 64% ± 2.4% of pre-STDP values, $F_{11.53,57.67}$ = 11.09, p < 0.0001, non-MD + NaB, n = 6: 75% ± 3% of pre-STDP values, $F_{15.23,45.69}$ = 2.436, p < 0.01; unpaired Student's t tests: p < 0.001 for LTD levels between non-MD and non-MD + NaB; Figure 8C, non-MD + DMSO, n = 5: 60% ± 3.1% of pre-STDP values, $F_{11.35,22.7}$ = 6.087, p < 0.0001, non-MD + CI-994, n = 4: 99% ± 0.6% of pre-STDP values, $F_{30.9,92.75}$ = 1.266, p = 0.243; Figure 8E, STD-LTP$_{GABA}$: non-MD, n = 8: 151% ± 8.4% of pre-STDP values, $F_{5.26,36.87}$ = 3.369, p = 0.012, non-MD + NaB, n = 9: 117% ± 11% of pre-STDP values, $F_{4.74,9.485}$ = 0.778,

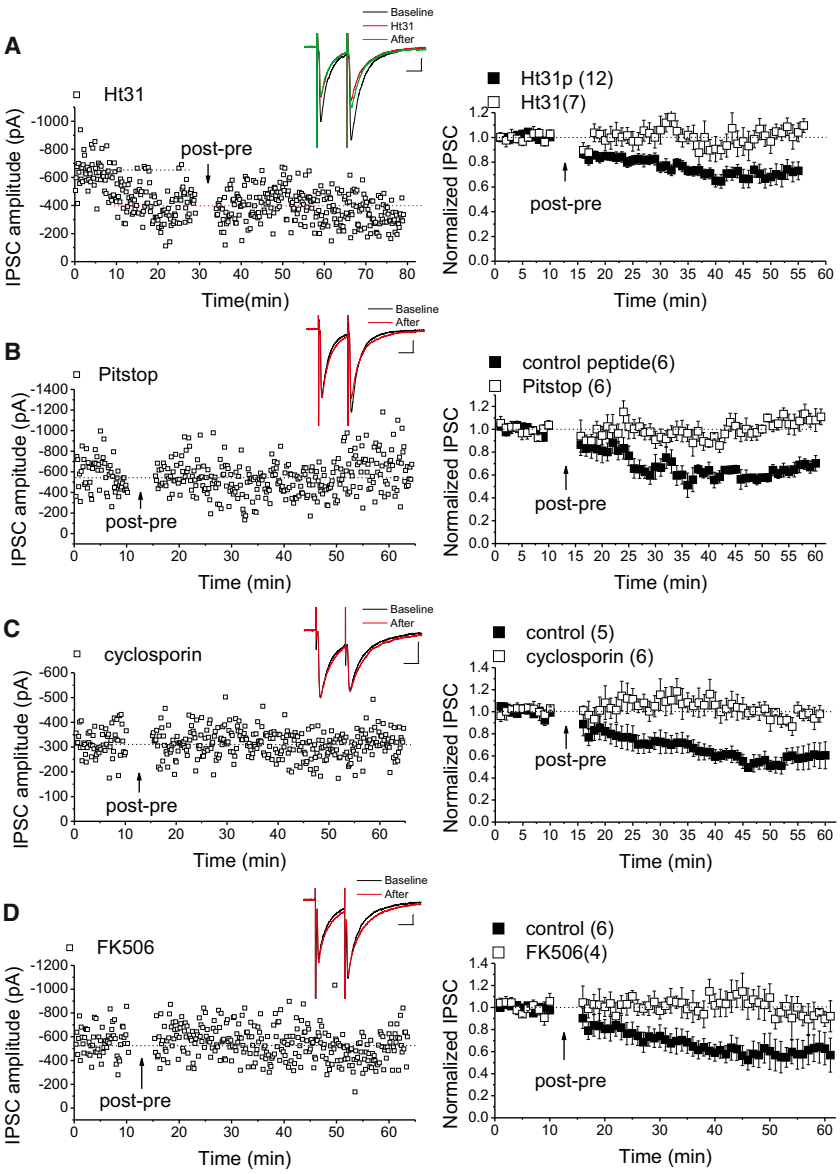

Figure 5. STD-LTD$_{GABA}$ Was AKAP and CaN Dependent and Required Clathrin-Mediated Internalization of GABA$_A$ Receptors for Its Expression

(A–D) Only non-MD rats were used for this set of experiments. (A) Single experiment (left) illustrating the effect of intra-pipette Ht31 on basal synaptic transmission and the induction of STD-LTD$_{GABA}$ in the Ht31 loaded cell (only the last 5 min of the baseline responses before the emergence of the Ht31-induced depression is shown in this sample experiment). Inset: averaged IPSCs before (black) or the peak response of Ht31 (red) or 25 min after post-pre protocol (green). Calibration: 100 pA, 25 ms. Averaged STD-LTD$_{GABA}$ experiments (right) with intra-pipette Ht31 (open symbols) or Ht31p (filled symbols). (B) Single experiment (left) illustrating the block of STD-LTD$_{GABA}$ by intra-pipette Pitstop2. Inset: averaged IPSCs before (black) or 25 min after post-pre protocol (red). Calibration: 100 pA, 25 ms. Averaged STD-LTD$_{GABA}$ experiments (right) with Pitstop2 control peptide (filled symbols) or Pitstop2 (open symbols). (C and D) Single experiments (left) illustrating the block of STD-LTD$_{GABA}$ by intra-pipette CaN inhibitors cyclosporin A and FK506. Insets: averaged IPSCs before (black) or 25 min after post-pre protocol (red). Calibration: 100 pA, 25 ms. Averaged experiments (right) with (open symbols) and without (filled symbols) 1 μM CaN inhibitor in the pipette solution. Error bars represent SEM.

p = 0.583; Figure 8G, non-MD + DMSO, n = 6: 139% ± 5.8% of pre-STDP values, $F_{21.59,86.39}$ = 5.022, p < 0.0001 non-MD + CI-994, n = 5: 119% ± 2.4% of pre-STDP values, $F_{8.49,25.48}$ = 1.23, p = 0.332).

To confirm whether the HDACi-mediated reversal of GABAergic metaplasticity induced by MD is due to normalization of AKAP signaling by HDAC inhibition, we tested the effects of HDACis (NaB and CI-994) on Ht31-induced depression in slices from non-MD and MD rats. HDACi incubation also restored the Ht31-induced rundown of IPSCs in midbrain slices from MD animals (Figure 6C, MD + Ht31, n = 9: 91% ± 5.8% of the first 5-min baseline values, $F_{5.468,32.808}$ = 1.442, p = 0.232; MD + Ht31 + NaB, n = 11: 60% ± 2.8% of the first 5-min baseline values, $F_{4.596,13.787}$ = 13.77, p < 0.0001; Figure 6E, MD + Ht31 + DMSO, n = 5: 99.5% ± 2.5% of the first 5-min baseline values, $F_{4.54,13.63}$ = 0.58, p = 0.701; MD + Ht31 + CI-994, n = 6: 65% ± 1.3% of the first 5-min baseline values, $F_{17.97,84.86}$ = 15.133, p < 0.0001). Ht31-induced run-down remained unchanged after NaB or CI-994 treatment in slices from non-MD rats (Figure 6B, non-MD + Ht31, n = 7: 67% ± 2.8% of the first 5-min baseline values, $F_{4.36,13.079}$ = 13.938, p < 0.0001; non-MD + Ht31 + NaB, n = 6: 68% ± 1.3% of the first 5-min baseline values, $F_{6.76,33.82}$ = 11.517, p < 0.0001; Figure 6D, non-MD + Ht31 + DMSO, n = 6: 64.9% ± 1.4% of the first 5-min baseline values, $F_{3.85,15.42}$ = 12.973, p < 0.0001; non-MD + Ht31 + CI-994, n = 4: 56% ± 1.4% of the first 5-min baseline values, $F_{11.2,22.3}$ = 4.999, p < 0.0001).

Given that local HDAC inhibition (through two structurally different HDACis) was sufficient to reverse metaplastic abnormalities of GABAergic transmission, we hypothesized that sustained HDAC-mediated downregulation of AKAP150 gene expression may occur in VTA DA neurons following MD. A decrease in the availability of AKAP proteins at GABAergic synapses in the VTA could promote less confinement of GABA$_A$Rs at the synapse, thereby favoring LTD (MD-induced metaplasticity). To test this hypothesis, we performed a double-immunofluorescence staining technique using antibodies against TH (marker for DA neurons) and AKAP150, allowing us to visualize DA neurons expressing AKAP150. Surprisingly, we detected higher levels of

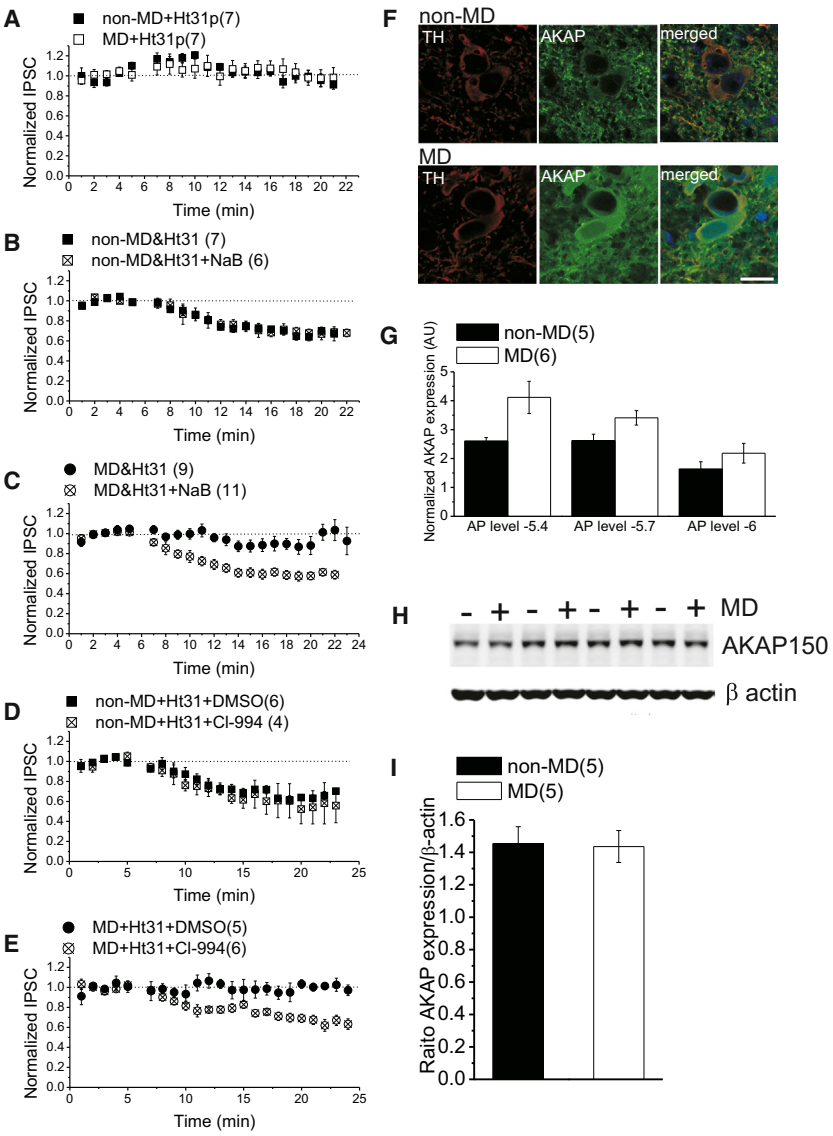

Figure 6. MD Induced Reversible HDAC-Mediated Disruption in AKAP150 Signaling in VTA DA Neurons

(A) Averaged experiments with intra-pipette 1 μM Ht31p on $GABA_A$ IPSCs recorded from DA neurons in slices from non-MD rats (filled symbols) or MD rats (open symbols).

(B) Averaged experiments with intra-pipette 1 μM Ht31 (filled symbols) or intra-pipette Ht31 + in vitro HDACi incubation (either NaB or CI-994, crossed symbols) on $GABA_A$ IPSCs recorded from DA neurons in slices from non-MD rats.

(C) Averaged experiments with intra-pipette 1 μM Ht31 (filled symbols) or intra-pipette Ht31 + in vitro HDACi incubation (NaB or CI-994, crossed symbols) on $GABA_A$ IPSCs recorded from DA neurons in slices from MD rats.

(D) Averaged experiments with intra-pipette 1 μM Ht31 (filled symbols) or intra-pipette Ht31 + in vitro HDACi incubation (either NaB or CI-994, crossed symbols) on $GABA_A$ IPSCs recorded from DA neurons in slices from non-MD rats.

(E) Averaged experiments with intra-pipette 1 μM Ht31 (filled symbols) or intra-pipette Ht31 + in vitro HDACi incubation (NaB or CI-994, crossed symbols) on $GABA_A$ IPSCs recorded from DA neurons in slices from MD rats.

(F) Examples of brain sections stained with antibodies to TH (red), AKAP150 (green), and DAPI (blue) with the merged panels, which show the expression of AKAP150 in TH neurons in the VTA of non-MD (top) and MD (bottom) rats. Scale bar, 20 μm.

(G) Averaged levels of AKAP150 expression at three AP levels from non-MD and MD rats.

(H and I) Representative western blots (top) and quantitative data (bottom) of AKAP 150 and β-actin (control) in VTA homogenates from non-MD and MD rats.

Error bars represent SEM.

AKAP immunoreactivity in TH-positive cells of MD compared to non-MD rats at three AP levels within the VTA (−5.4, −5.7, and −6 mm caudal to bregma) (Figures 6F and 6G, non-MD, n = 5; MD, n = 6; two-way ANOVA, p = 0.0016). To further determine AKAP expression in the VTA, we performed western blots of VTA homogenates isolated from non-MD and MD rats. There was no significant difference in AKAP expression in the VTA between non-MD and MD rats (Figures 6H and 6I, non-MD, n = 5; MD, n = 5, unpaired Student's t test, p = 0.9056). Thus, the enhanced AKAP immunofluorescence may be related to translocation of AKAP away from the synapse, uncoupling AKAP from $GABA_A$Rs. Together, our data provide the first experimental evidence linking the scaffolding protein, AKAP150, to induction of metaplasticity by severe early-life stress at inhibitory synapses. The restoration of normal levels of GABAergic transmission and STDP by HDACis in slices from MD animals is achieved locally in the VTA through epigenetic rescue of AKAP signaling to VTA DA neurons.

DISCUSSION

Our study demonstrated that severe early-life stress may promote the dysregulation of DA signaling and reward circuitry through an epigenetic-mediated GABAergic metaplasticity in the VTA. This epigenetic modification involved the postsynaptic scaffolding protein AKAP150 and was potentially reversible if targeted during the early stages following trauma.

A number of studies have found a strong link between the dysregulation of DA signaling by early postnatal life stress such as child maltreatment and a later propensity to develop stress-related disorders and addiction. This link raises the possibility that alterations in VTA DA neuronal activity and synaptic function may occur following childhood trauma (Rentesi et al., 2013). Moreover, childhood adversities trigger dysfunction in epigenetic control of neuronal activity and synaptic plasticity, thus having a significant impact on the brain and behavior (Rodenas-Ruano et al., 2012). Histone modifications are induced by MD through HDAC activity (Levine et al., 2012; Tesone-Coelho et al., 2015; Valvassori et al., 2014), suggesting the potential therapeutic benefits of early-life epigenetic intervention. These observations led us to hypothesize that early-life adversity engages

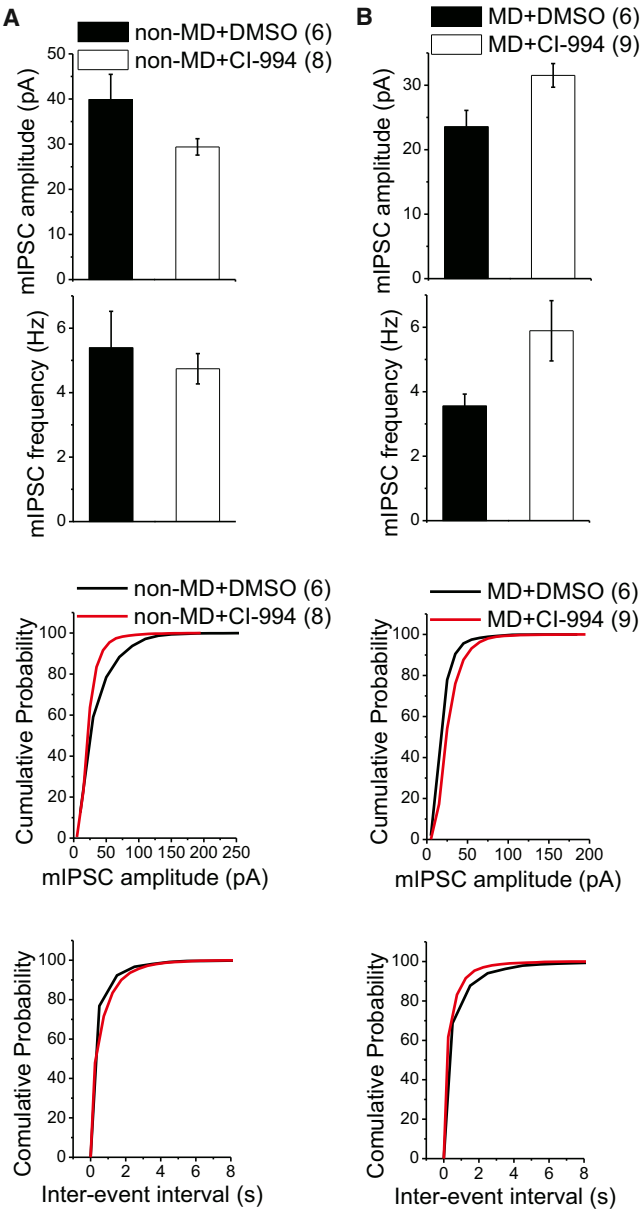

Figure 7. MD-Induced GABAergic LTD Was Reversed by HDAC Inhibition

(A) Average bar graphs and cumulative probability plots of mIPSC amplitude and frequency from non-MD rats with DMSO (vehicle) and CI-994.

(B) Average bar graphs and cumulative probability plots of mIPSC amplitude and frequency from MD rats with DMSO (vehicle) and CI-994.

Error bars represent SEM.

epigenetic mechanisms regulating synaptic activity and learning mechanisms in VTA DA neurons and that these synaptic maladaptations may be reversed by HDACis if targeted soon after the insult. HDACis have proved effective in attenuating neurodegenerative-associated cognitive declines and fear-related memories by affecting epigenetic mechanisms involved in neuronal plasticity (Gräff et al., 2012, 2014). Here, we provide the first evidence that GABAergic synaptic abnormalities occur in the VTA in response to an episode of prolonged severe early-life stress (i.e., MD). In addition, our data suggest that these abnormalities are at least partly mediated through epigenetic modifications of genes involved in the AKAP signaling complex. Previously, we reported that AKAP150 signaling plays a selective role in regulating a D2 dopamine receptor (D2R)-mediated CaN-dependent GABAergic LTD. We found that disruption of PKA-AKAP association mimics and occludes this LTD without altering glutamatergic transmission in VTA DA neurons (Dacher et al., 2013; Dacher and Nugent, 2011a). Remarkably, AMPAR-mediated glutamatergic transmission and subunit composition of synaptic AMPARs were also unaffected after early MD while GABAergic transmission and plasticity in DA neurons were significantly impacted by MD. This is consistent with the findings that maternal care significantly influences the epigenetic regulation of GABAergic signaling in the brain (Zhang et al., 2010). Some of our observations here implicate MD-induced GABAergic metaplasticity in VTA DA neurons through signaling modifications that involve AKAP150. Inhibition of PKA-AKAP interaction inhibited STD-LTP$_{GABA}$ and occluded further induction of STD-LTD$_{GABA}$, suggesting that AKAP-anchored PKA and CaN also underlie postsynaptic STDP. Although MD per se induced a postsynaptic LTD (suggested by the reduced amplitude of mIPSCs, indicative of postsynaptic modifications) and occluded STD-LTD$_{GABA}$ in response to post-pre protocol, MD changed the learning rules of bidirectional Hebbian STDP at GABAergic synapses in the VTA to enable LTD. We found that GABAergic synapses of MD rats exhibited LTD in response to pre-post pairing protocol at +5 ms, while non-MD rats did not show any form of GABAergic STDP. Moreover, MD not only prevented the induction of STD-LTP$_{GABA}$ in response to pre-post pairing at +15 ms but also shifted the polarity of this plasticity toward LTD. MD-induced LTD in response to pre-post protocols at +15 ms was CaN dependent. More importantly, CaN blockade did not rescue STD-LTP$_{GABA}$. The fact that LTP was not present even after the blockade of CaN-dependent LTD confirms that the induction of LTP was inhibited by MD and not simply masked by MD-induced LTD. Thus, MD-induced metaplasticity facilitated CaN-dependent STD-LTD$_{GABA}$ while inhibiting STD-LTP$_{GABA}$.

Postsynaptic AKAP inhibition was insufficient to trigger chemical LTD in MD slices, suggesting that disruption of AKAP signaling by MD may be the mechanistic link for MD-induced metaplasticity and synaptic deficits. Local HDAC inhibition in the VTA was sufficient to reverse metaplastic abnormalities of GABAergic transmission by MD. However, MD did not change the levels of AKAP150 protein in VTA extracts, suggesting that MD disrupted AKAP signaling without transcriptional repression of the AKAP150 gene. Interestingly, we detected higher levels of AKAP immunoreactivity in VTA DA neurons in MD pups. Thus, MD likely dysregulated normal AKAP postsynaptic targeting at GABAergic synapses onto VTA DA neurons, which led to the translocation of AKAP or AKAP-interacting proteins away from the synapse. AKAP complexes are not stationary and can be redistributed dynamically in response to stimuli such as NMDAR activation or rises in intracellular Ca^{2+} (Gomez et al., 2002; Smith et al., 2006). Consistent with this hypothesis, NMDAR-dependent glutamatergic LTD in the hippocampus

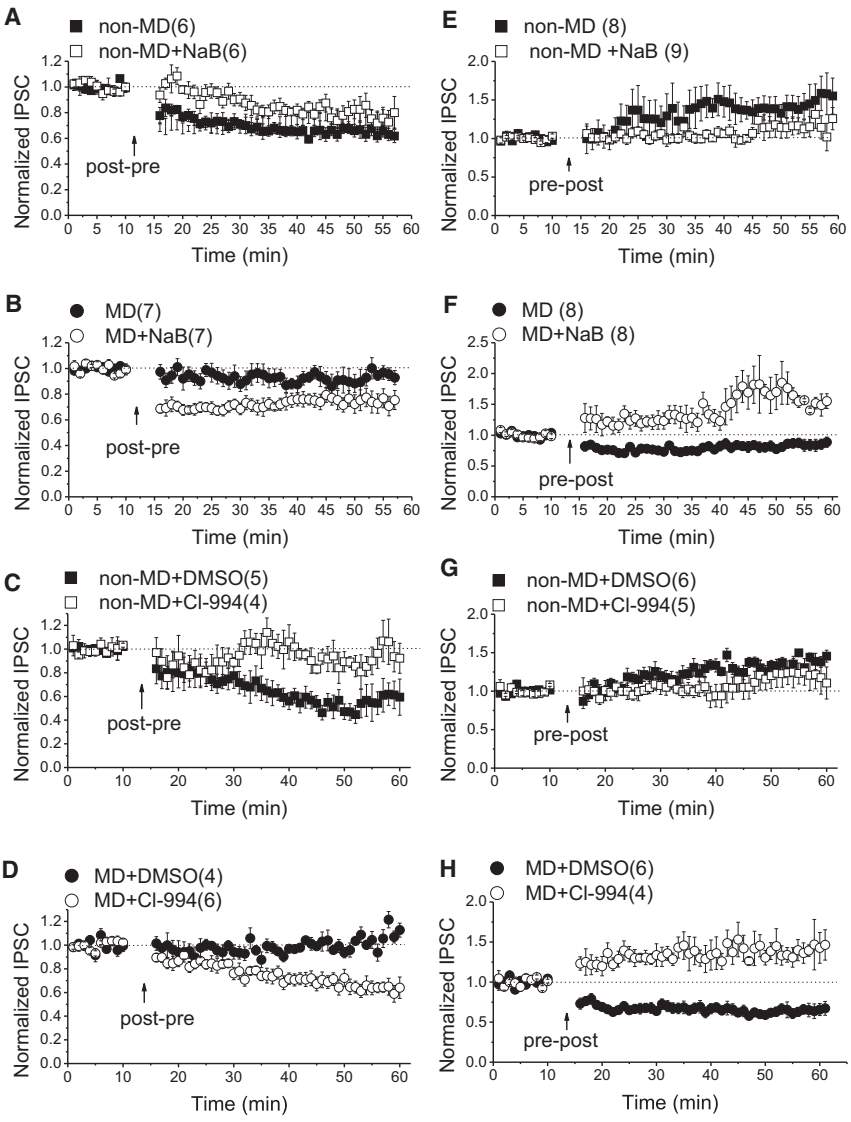

Figure 8. MD-Induced GABAergic Metaplasticity Was Reversed by HDAC Inhibition

(A–H) Error bars represent SEM. Shown are averaged STD-LTD$_{GABA}$ experiments with (open symbols) or without (filled symbols) HDACi (either NaB or CI-994) treatment in slices from non-MD rats (A and C), averaged STD-LTD$_{GABA}$ experiments with (open symbols) or without (filled symbols) HDACi (NaB or CI-994) treatment in slices from MD rats (B and D), averaged STD-LTP$_{GABA}$ experiments with (open symbols) or without (filled symbols) HDACi (either NaB or CI-994) treatment in slices from non-MD rats (E and G), and averaged STD-LTP$_{GABA}$ experiments with (open symbols) or without (filled symbols) HDACi (either NaB or CI-994) treatment in slices from MD rats (F and H).

involves a CaN-dependent translocation of AKAP150 and its anchored pool of PKA away from synapses. This in turn uncouples AKAP-PKA complexes from CaN-dephosphorylated AMPARs and prevents their subsequent re-phosphorylation by PKA (Smith et al., 2006). AKAP150 is also required for PKA phosphorylation of GABA$_A$Rs in the hippocampus (Brandon et al., 2003), although it is unknown how AKAP150 signaling complexes are formed at GABAergic synapses and coordinate the opposing functions of kinases and phosphatases under physiological and pathological conditions. The interaction between AKAP150 and the GABA$_A$R subunits also occurs in the Golgi apparatus (Lilly et al., 2005), which raises the possibility that MD may have triggered a redistribution of AKAP that influenced the subcellular localization and anchoring of PKA and CaN at GABAergic synapses. Therefore, the rescue of normal AKAP signaling and GABAergic plasticity in slices from MD rats by HDACis may occur through the reversal of HDAC-mediated transcriptional changes in the expression of AKAP-interacting proteins or signaling molecules upstream of AKAP that are necessary for proper functioning, interaction, or localization of AKAP at the synapse.

Similar to what we observed with MD animals, postsynaptic inhibition of PKA alone is sufficient to shift the polarity of STDP toward LTD in response to pre-post firing, suggesting that changes in PKA activity downstream of neuromodulators such as DA may be a critical determinant for the direction of STDP. Given that D2R stimulation through PKA inhibition promotes postsynaptic LTD$_{GABA}$ in VTA DA neurons (Dacher et al., 2013), it will not be surprising that part of MD-induced facilitation of LTD and disruption of AKAP signaling involve reversible epigenetic changes in DA-D2R-PKA mediated signaling that facilitates CaN activity. Although the levels of NMDAR activation and the ensuing increase in intracellular Ca^{2+} are considered the main determinants of the polarity of synaptic changes, neuromodulators including DA can control this polarity and change the rules of STDP induction (Pawlak et al., 2010). Therefore, it will be worthwhile to investigate whether MD increases dendrodendritic release of DA in the VTA and promotes D2R-mediated modulation of GABAergic metaplasticity involving AKAP. In a scenario where dendrodendritic DA release is elevated by MD, this can also lead to presynaptic inhibition of GABA release from GABAergic terminals onto DA neurons (suggested by the decrease in the frequency of IPSCs in MD slices, which is also recovered by HDAC inhibition in our experiments) through activation of presumably presynaptic D2Rs and engaging endocannabinoid signaling and presynaptic cAMP-PKA pathway enabling a presynaptic LTD (Pan et al., 2008a, 2008b).

MD-induced GABAergic synaptic modifications could shift the balance of glutamatergic/GABAergic synaptic inputs onto DA neurons toward a decreased GABAergic inhibition, increasing

DA neuronal excitability and DA release. In our STDP experiments, postsynaptic firing is controlled by direct somatic current injections through patch pipettes while holding the cells at −65 mV to avoid random postsynaptic firing. It is plausible to assume that the increase in excitability of DA neurons in vivo by MD could lead to irregular and random pre-post and post-pre firing pairs. The polarity of plasticity would then favor LTD, as the direction of synaptic plasticity induced in response to more complex STDP protocol is toward LTD (Kodangattil et al., 2013).

In summary, we demonstrated that MD may alter motivated- and reward-related learning later in life partly through epigenetic modifications that disrupt AKAP signaling and trigger GABAergic metaplasticity in the VTA. MD-induced metaplastic and epigenetic changes in the learning rules governing spike timing GABAergic plasticity of VTA DA neurons may reinforce association of irrelevant synaptic inputs, which would have not been correlated under physiological conditions, thereby falsely exaggerating the incentive salience of stimuli such as drug-associated cues. Further mechanistic and epigenetic studies of early-life stress-induced neuroplasticity within the VTA are warranted to identify novel signaling cascades in regulation of DA signaling and reward learning.

EXPERIMENTAL PROCEDURES

Male juvenile Sprague-Dawley rats were used for all studies. All procedures were performed according to approved protocols by the Uniformed Services University Animal Care and Use Committee in accordance with the guidelines set forth by the National Institutes of Health. Detailed experimental procedures can be found in Supplemental Experimental Procedures.

Maternal Deprivation Procedure

For 24-hr MD, five rat pups (half of the pups from normal litters) at P9 were isolated at 10:00 a.m. from the dam and siblings and placed in a separate cage with a heating pad (34°C) located in another room to avoid disturbance by vocalization in the original animal room (MD group) as described previously (Ellenbroek et al., 2005). The pups were not disturbed and were returned to the home cage after 24 hr. The remaining non-separated rat pups (five pups) received the same amounts of handling and served as the non-maternally deprived control group (non-MD group). Each day, two rats (age-matched MD and non-MD rats of the same litter) were sacrificed over P14–P21 for electrophysiology, immunohistochemistry, and western blotting. Only one cell per animal (non-MD or MD) was recorded to demonstrate that the MD effects are consistently observed across multiple animals; therefore, all reported n values represent the number of animals recorded.

Slice Preparation for Electrophysiology

Horizontal midbrain slices (250 μM) were prepared from 14- to 21-day old rats. Slices were cut and incubated for at least 1 hr at 34°C before recording in artificial cerebrospinal fluid (ACSF) containing 126 mM NaCl, 21.4 mM $NaHCO_3$, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.0 mM $MgSO_4$, 11.1 mM glucose, and 0.4 mM ascorbic acid, saturated with 95% O_2/5% CO_2. Slices were then transferred to recording chambers and submerged in warm (28°C), ascorbic acid-free ACSF.

Electrophysiology

$GABA_A$ receptor-mediated IPSCs were evoked, isolated, and recorded in ACSF containing 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM) and strychnine (1 μM). For mIPSC recordings, tetrodotoxin (TTX, 1 μM) was also added to the perfusion bath. The patch pipettes (3–6 cMΩ) were filled with 125 mM KCl, 2.8 mM NaCl, 2 mM $MgCl_2$, 2 mM ATP-Na^+, 0.3 mM GTP-Na^+, 0.6 mM EGTA, and 10 mM HEPES (pH adjusted to 7.28 with KOH, osmolarity adjusted to 275–280 mOsm). Cells were voltage-clamped at −70 mV. Paired $GABA_A$ IPSCs were stimulated at 0.1 Hz (duration 100 μs, 50 ms inter-stimulation interval). To induce STDP, cells were taken to current clamp and received trains of a presynaptic stimulation paired with back-propagating action potentials at 5 Hz. STDP protocols consisted of 30 trains of five bursts repeated at 0.1 Hz. For STD-LTP_{GABA}, each burst was composed of three bAPs at 50 Hz and preceded by a single presynaptic stimulation (positive timing, +15 ms). For STD-LTD_{GABA}, each burst was composed of three bAPs at 50 Hz followed by a single presynaptic stimulation (negative timing, −5 ms).

Combined excitatory postsynaptic currents (EPSCs) were evoked and recorded in the presence of ACSF containing picrotoxin (100 μM) at +40 mV. Patch pipettes were filled with 117 mM Cs-gluconate, 2.8 mM NaCl, 5 mM $MgCl_2$, 2 mM ATP-Na^+, 0.3 mM GTP-Na^+, 0.6 mM EGTA, and 20 mM HEPES (pH adjusted to 7.28 with CsOH, osmolarity adjusted to 275–280 mOsm). APV (50 μM) was added to obtain AMPAR-only EPSCs at +40 mV. For AMPA receptor-mediated mEPSC recordings, APV (50 μM) and TTX (1 μM) were also added to ACSF that contained picrotoxin, and cells were voltage-clamped at −70 mV. For AMPAR rectification, AMPAR EPSCs were pharmacologically isolated and evoked as described previously (Sanderson et al., 2012) in the presence of ACSF containing 50 μM APV and 100 μM picrotoxin and inclusion of 10 μM spermine in the patch pipette using the Cs-based internal. AMPAR EPSCs were then recorded at different holding potentials (> 10/neuron), and normalized current-voltage (I-V) curves were generated by dividing the AMPAR EPSC peak amplitudes by the mean of AMPAR EPSC peak amplitude at −65 mV. AMPAR rectification was determined by dividing the peak amplitudes of AMPAR EPSCs recorded at −65 mV by those recorded at +40 mV for each neuron.

Western Blots

The VTA was dissected from midbrain slices of non-MD and MD rat pups and lysed in 1% RIPA buffer containing protease inhibitors. Protein extracts were prepared, run on SDS-PAGE gels, transferred, and probed as described in Supplemental Experimental Procedures. The primary antibody was anti-AKAP-150.

Immunohistochemistry and Image Analysis

Non-MD and MD rat pups were perfused with 4% paraformaldehyde, and coronal sections (20 μm) of the midbrain from MD and non-MD rat pups containing the VTA (from −4.92 to −6.72 mm caudal to bregma) were dissected. Sections were incubated overnight with TH and AKAP150 antisera, followed by incubation with appropriate secondary antisera as described in Supplemental Experimental Procedures.

Data Analysis

Values are presented as means ± SEM. Statistical significance was determined using repeated-measures ANOVA with significance level of $p < 0.05$ for STDP and chemical LTD ($PKI_{(6-22)}$ or Ht31) experiments. Unpaired Student's t tests were performed subsequent to significant repeated-measure ANOVAs for those experiments to make comparisons between MD and non-MD rats. Levels of STDP are reported as averaged IPSC amplitudes for 5 min just before STDP induction compared with averaged IPSC amplitudes during the 5-min period from 35 to 40 min after the protocol. The magnitude of STDP in Figure 3 was expressed as percentage of synaptic change. Levels of $PKI_{(6-22)}$-/Ht31-induced depression are reported as averaged IPSC amplitudes for 5 min just before the emergence of drug-induced synaptic depression compared with averaged IPSC amplitudes during the last 5-min period of the peak response. The statistical differences for AMPAR/NMDAR ratio and AMPAR EPSC rectification index were evaluated using unpaired Student's t test. Two-way ANOVA with Bonferroni post hoc analysis was used to detect the statistical difference between non-MD and MD rats for AMPAR EPSC IV plots. Unpaired Student's t test was used to analyze the statistical differences in the means of amplitude and frequency of mEPSCs and mIPSCs in the represented bar graphs. The KS test was performed for the statistical analyses of cumulative probability plots of mEPSCs and mIPSCs. MD effects on AKAP intensity were compared by a two-way ANOVA and Bonferroni post hoc analysis. AKAP expression was normalized to β-actin expression in western blot analysis, and an unpaired Student's t test was used to evaluate the statistical difference between the two groups.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.05.024.

AUTHOR CONTRIBUTIONS

F.S.N. designed the research; M.E.A., J.N.K., S.G., M.R., and F.S.N. performed the experiments; M.E.A., J.N.K., S.G., M.R., A.J.S., B.M.C., and F.S.N. analyzed the data and prepared the figures; M.E.A., J.N.K., S.G., A.J.S., B.M.C., and F.S.N. wrote the paper.

ACKNOWLEDGMENTS

The opinions and assertions contained herein are the private opinions of the authors and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences or the Department of Defense or Government of the United States. This work was supported by a Department of Defense intramural grant from the Uniformed Services University and the Brain and Behavior Research Foundation to F.S.N. The funding agencies did not contribute to writing this article or deciding to submit it. We thank Dr. Amanda Christy for her helpful suggestions on the design of animal studies. We are grateful to Drs. David Lovinger and Veronica Alvarez for their critical discussions for the present work.

Received: October 23, 2014
Revised: April 23, 2015
Accepted: May 7, 2015
Published: June 3, 2015

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